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VIABILITY OF PINE POLLEN STORED 15 YEARS[1/]

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Samples of pine pollen were collected at the Institute of Forest Genetics in 1945 and stored under varying relative humidities at 0° C. and 5° C. After 15 years, pollen which had been stored at 10 percent relative humidity at both 0°C. and 5°C., was still viable. Pollen samples stored under moisture conditions higher than 10 percent relative humidity were not viable. In samples of pollen stored at 10 percent relative humidity germination percent and tube length were higher in the pollen stored at 0°C. than in pollen stored at 5°C.

The relation between relative humidity and viability of pollen, including pine pollen, was first demonstrated by Pfundt in 1910. Holman and Brubaker (1926), Duffield and Snow (1941), and Johnson (1943) have applied the technique of storing pine pollen under atmospheres of constant relative humidity. The present report establishes the longest record of stored pine pollen retaining its capacity to germinate in vitro. It indicates that inexpensive storage conditions and non-freezing temperatures may be utilized for maintaining most pine pollen. However, the seed set and seedling development obtained when this stored pollen is used must be evaluated before final field recommendations can be made. Field pollination tests are now in progress.

Pollen used in this experiment was collected and extracted as described by Cumming and Righter (1948). Samples were placed in cotton stoppered test tubes, either 5 ml. or 20 ml. in volume, depending upon the quantity of pollen. The tubes were then placed in a one-pint Mason jar. An open 50 ml. vial of sulfuric acid, its density adjusted to maintain proper

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relative humidity according to the Handbook of Chemistry and Physics, was placed in the jar with the tubes of pollen. The jars were screwed tightly shut and placed in refrigerated boxes set at 0°C. and 5°C. At the end of 13 years (1958) germination counts were made. All jars were moved to 5°C. and maintained at that temperature for 2 years. The germination counts were repeated in 1959 and 1960. Data for 1960 are reported in table 1.

Germination counts were made on samples of approximately 20 mg. of pollen sprinkled on two drops of .002 M calcium phosphate buffer, pH 5.9, on a microscope slide placed in a moist Petri plate at 29° C. for 48 hours. One hundred grains were counted at random in each replicate drop. All pollen stored 13 years at 0°C. and 10 percent relative humidity and 2 years at 5°C. and 10 percent relative humidity showed some germination. Pollen of Pinus ponderosa was most viable at 77 percent germination, and pollen of P. radiata was least viable, at 5 percent (table 1). However, when the storage temperature was 5°C. for the entire 15 years, germination was nil in some samples and decreased in all samples except Pinus taeda.

Pinus radiata stored at 10 percent relative humidity gave poor, or no germination after both 1 year and 15 years storage (table 2). None of the pollen stored at or above 25 percent relative humidity germinated in the 15 year old samples. The increase in percent germination of Pinus lambertiana between 1 and 15 years is difficult to explain. None of the germination values obtained by Duffield (1954) on P. lambertiana pollen in 1946, after storage for 398 days in 10 percent relative humidity, approached the 45 percent value obtained in 1960. However, the methods of germination of pollen and size of the samples in 1946 tests and 1960 differed. Duffield obtained germination by permitting the pollen to slowly absorb moisture at 26 to 28°C. In a comparison of the vapor absorption and drop method, P. lambertiana stored at 10 percent relative humidity gave 66 percent germination in a vapor chamber and only 23 percent germination in a drop of water (Duffield, 1954). But pollen stored at 50 percent relative humidity gave equivalent germination by both methods, i.e. 70 versus 73 percent. Thus, the different germination procedures applied to the pollen may account for some of the observed differences in germination. Interestingly, Duffield's maximum of 31 percent germination for P. lambertiana obtained by the vapor method, followed storage at 22°C. and 10 percent relative humidity, not 0° or 5°C. storage. Storage at 25 percent relative humidity gave high germination at low storage temperatures, and a decrease or no germination at higher storage temperatures. The metabolism of endogenous carbohydrate substrates would be expected to increase at higher storage temperatures.

Duffield and Snow (1941) found that storage at 50 percent relative humidity was more favorable than storage at 0 percent for Pinus strobus and Pinus resinosa. Fechner, et al. (1960) reported that ponderosa pine pollen stored for 6 years was more viable when stored at 0 -4°C. and 50 percent relative humidity than at 0° or 25 percent relative humidity. Johnson (1943) and Fechner (1957) observed that storage humidities as high as 75 percent were beneficial for maintaining optimum germination of some conifer pollens. Thus, as one might expect, pollen of different species frequently behaves differently under similar storage conditions.

Pollen survival and viability after storage is probably most closely related to internal levels of growth substances and nutrient substrates.

Table 3 shows the relative tube lengths of the stored pollen after 48 hours germination. Maximum tube growth was attained by pollen which was most viable as measured by percent germination. As a comparison, the length of tube produced by fresh Pinus ponderosa pollen in 48 hours of germination under similar conditions is approximately 200 units.

Although storage of pine pollen at temperatures of -23°C. or -50°C. can also insure good germination (Manzhos, 1958; Callaham and Duffield, 1959), a general recommendation prompted by these in-vitro germination results is that pine pollen should at least be stored at 0°C. and 10 percent relative humidity.

Table 1.--Percent germination of pine pollen stored 15 years at varying relative humidities

Species	Storage temperature	Germination when storage relative humidity (percent) was--			
		10	25	50	75
	Degrees				
P. strobus	0 [1/]	53	0	0	0
P. canariensis	"	13	0	0	0
P. ponderosa	"	77	0	0	0
P. radiata	"	5	0	0	0
P. lambertiana	"	45	0	0	0
P. echinata	"	66	0	0	0
P. taeda	"	33	0	0	0
P. strobus	5	15	0	0	
P. canariensis	"	1	0	0	
P. ponderosa	"	58	0	0	
P. radiata	"	0	0	0	
P. lambertiana	"	26	0	0	
P. echinata	"	33	0	0	
P. taeda	"	36	0	0	

1/ 0°C. for 13 years, 5°C. for two years.

Table 2.--Mean percent germination of pine pollen collected in 1945, after approximately 1 year and 15 years[1/] storage at 0°C., 10 percent relative humidity

Species	Year of germination	
	[2/]1946	1960
P. strobus	85	53
P. canariensis	61	13
P. ponderosa	71	77
P. radiata	2	5
P. lambertiana	9	45
P. echinata	86	66
P. taeda	39	33

Sample size (n) 1946 = 100; germination of vapor method.
(n) 1960 = 200; germination by drop method.

1/ 0°C., 3 years, 5°C. for 2 years.
2/ Duffield (1954).

Table 3.--Mean tube lengths of pine pollen stored 15 years at 10 percent relative humidity and germinated 48 hours at 30°C.

Species	Storage temperature	
	[1/]0°C.	5°C.
P. strobus	36.2	33.5
P. canariensis	54.6	46.3
P. ponderosa	135.4	87.4
P. radiata	52.4	--
P. lambertiana	52.0	44.7
P. echinata	52.2	56.9
P. taeda	42.1	46.9

Sample size (n) = 200; units approximate microns.
1/ 0°C., for 13 years, 5°C. for 2 years.

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